Detection of Insulin in Formalin-Fixed, Paraffin-Embedded, Rodent and Human Pancreas

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer Antigen Retrieval

Antibody Dilutions:

Block, Link and Label Antibody: Use the Vectastain Guinea pig IgG ABC Kit (Catalog # PK 4007). Prepare the blocking, link and label antibody according to the manufacture's suggestion. An instruction sheet is provided within each kit.

Primary Antibody
Rodents (Rats and Mice) and Humans
Polyclonal Antibody: Guinea pig anti-porcine insulin (A564)
Dilution: 1:3000
A564 recognizes beta cells in the Islets of Langerhans in the pancreas. The antibody cross-reacts with insulin from many mammals.
Dako Corporation
Catalog #: A564
800-235-5763

Staining Procedure

This staining procedure is for tissues fixed in 10% neutral buffered formalin and embedded in paraffin.

Positive Control Tissue: Pancreas ->Islets of Langerhans

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.
2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
3. Place slides in plastic Coplin jars containing retrieval solution, citrate buffer. Jars are covered with loose-fitting screw caps and heated in the MWO on high (~750 watts) for 5 minutes. To prevent boiling over and excessive evaporation of the retrieval solution, it is advised to irradiate 3 staining jars simultaneously. If the jars do not contain slides they are used as a water load. After the first 5 minute MWO cycle, check the fluid level of all jars. If the level of citrate buffer or water is low (below the tissue section), discard the retrieval solution and add fresh solution to the container.
4. After heating remove jars from the MWO and check the fluid levels. Discard old retrieval solution and add more solution. Allow to cool at room temperature for 15 minutes.
5. Rinse slides in distilled water twice and then in 1X Automation Buffer for 5 minutes.
6. Apply the blocking antibody for 20 minutes at room temperature. DO NOT RINSE SECTIONS WITH BUFFER BEFORE APPLYING PRIMARY ANTIBODY.
7. Apply insulin primary antibody and incubate for 30 minutes at room temperature. A control slide should be processed and incubated with the control antibody, normal guinea pig serum.
8. Rinse in 2 changes of 1X Automation Buffer for 5 minutes each.
9. Apply the link antibody. Incubate for 30 minutes at room temperature.
10. Rinse in 2 changes of 1X Automation Buffer for 5 minutes each.
11. Apply the label antibody. Incubate for 30 minutes at room temperature.
12. Rinse in 2 changes of 1X Automation Buffer for 5 minutes each.
13. Proceed with chromogen of choice, AEC or DAB.

Antigenic sites: positive cytoplasmic staining updated 4/8/03